Claims

1. A method for determining the prognosis of a subject with a cell proliferative disorder of the breast tissues, said method comprising analysing the methylation pattern of a target nucleic acid comprising one or a combination of the genes taken from the group consisting of ESR1, APC, HSD174B4, HIC1 and RASSF1A and/or their regulatory regions by contacting at least one of said target nucleic acids in a biological sample obtained from said subject with at least one reagent, or series of reagents that distinguishes between methylated and non-methylated CpG dinucleotides.

2. A method for selecting a treatment and/or for monitoring a treatment of a cell proliferative disorder of the breast tissues, said method comprising:
   a) determining the prognosis of a subject according to Claim 1, and
   b) selecting a suitable treatment according to said prognosis and/or monitoring the treatment success according to said prognosis.

3. The method of claim 2, wherein said suitable treatment is a hormonal/antihormonal therapy, a chemotherapy and/or an adjuvant therapy.

4. The method of claim 3, wherein said suitable treatment is a hormonal/antihormonal therapy and wherein the determination of said prognosis comprises the analysis of the methylation pattern of a target nucleic acid comprising the RASSF1A gene and/or its regulatory region(s).

5. The method of claim 3 or 4, wherein said hormonal/antihormonal therapy comprises a tamoxifen therapy.

6. The method of claim 5, wherein persistence, increase, appearance or re-appearance of RASSF1A methylation indicates a resistance to tamoxifen treatment and/or wherein a decrease or disappearance of RASSF1A methylation is indicative for a response to tamoxifen treatment.

7. A method for determining the phenotype of a subject with a breast cell proliferative disorder comprising

a) obtaining a biological sample containing genomic DNA from said subject,

b) analysing the methylation pattern of one or more target nucleic acids comprising one or a combination of the genes taken from the group consisting of ESR1, APC, HSD174B4, HIC1 and RASSF1A and/or their regulatory regions by contacting at least one of said target nucleic acids in the biological sample obtained from said subject with at least one reagent, or series of reagents that distinguishes between methylated and non-methylated CpG dinucleotides, and

c) determining the phenotype of the individual by comparison to two known phenotypes, a first phenotype characterised by hypermethylation of the target nucleic acid and poor prognosis as relative to a second phenotype characterised by hypomethylation of the analysed target nucleic acid and positive prognosis.

8. A method according to claims 1 to 3, 7 and 8 wherein said prognosis is the life expectancy of said subject or wherein said prognosis is the treatment success of a cell proliferative disorder of the breast tissues.

9. A method according to any one of claims 1 to 3, 7 and 8 wherein said target nucleic acid comprises the gene APC and/or its regulatory regions.

10. A method according to any one of claims 1 to 8 wherein said target nucleic acid comprises the gene RASSF1A and/or its regulatory regions.

11. A method according to any one of claims 1 to 8 wherein said target nucleic acids comprise the genes APC and RASSF1A and/or their regulatory regions.

12. A method according to any one of claims 1 to 11, wherein said target nucleic acid or acids comprise essentially one or more sequences from the group consisting of SEQ ID NOs: 1 to 5 and sequences complementary thereto.

13. A method according to claim 9 wherein the sequence of said target nucleic acid is or comprises the nucleic acid molecule of SEQ ID NO: 3 or a fragment thereof.

14. A method according to claim 10 wherein the sequence of said target nucleic acid is or comprises the nucleic acid molecule of SEQ ID NO: 5 or a fragment thereof.

15. A method according to claim 11, wherein said target nucleic acid or acids is or comprises the nucleic acid molecule as shown in SEQ ID NOs: 3 and 5 or a fragment of said nucleic acid molecules.

16. A method according to any one of claims 1 to 15, wherein said cell proliferative disorder of the breast tissue is selected from the group consisting of ductal carcinoma in situ, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metaplastic carcinoma, intraductal carcinoma in situ, lobular carcinoma in situ and papillary carcinoma in situ.

17. A method according to any one of claims 1 to 16, wherein said biological sample is a blood sample, serum or NAF (nipple aspirate fluid).

18. A nucleic acid molecule consisting essentially of a sequence at least 18 bases in length according to one of the sequences taken from the group consisting of SEQ ID NOs: 6 to 25 and sequences complementary thereto.

19. An oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, said oligomer consisting essentially of at least one base sequence having a length of at least 10 nucleotides which hybridises to or is identical to one of the nucleic acid sequences according to SEQ ID NOs: 6 to 25.

20. The oligomer as recited in any one of claims 18 or 19 , wherein the base sequence includes at least one CpG dinucleotide.

21. A set of oligomers, comprising at least two oligomers according to any of claims 18 or 19.

22. A set of oligonucleotides as recited in claim 21, characterised in that at least one oligonucleotide is bound to a solid phase.

23. A set of at least two oligonucleotides as recited in any of claims 19 or 20, which is used as primer oligonucleotides for the amplification of nucleic acid sequences comprising one of SEQ ID NOs: 6 to 25 and sequences complementary thereto.

24. Use of a set of oligonucleotides comprising at least two of the oligomers according to any one of claims 21 to 23 for detecting the cytosine methylation state and/or single nucleotide polymorphisms (SNPs) within the sequences taken from the group SEQ ID NOs: 1 to 5 and sequences complementary thereto.

25. A method for manufacturing an arrangement of different oligomers (array) fixed to a carrier material for predicting the responsiveness of a subject with a cell proliferative disorder of the breast tissues by analysis of the methylation state of any of the CpG dinucleotides of the group SEQ ID NOs 1 to 5 wherein at least one oligomer according to any of the claims 19 or 20 is coupled to a solid phase.

26. An arrangement of different oligomers (array) obtainable according to claim 25.

27. An array of different oligonucleotide- and/or PNA-oligomer sequences as recited in claim 26, characterised in that said oligonucleotides are arranged on a plane solid phase in the form of a rectangular or hexagonal lattice.

28. The array as recited in any of the claims 26 or 27, characterised in that the solid phase surface is composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold.

29. A DNA- and/or PNA-array for predicting breast cell proliferative disorders' response by analysis of the methylation state of any of the CpG dinucleotides of the group SEQ

ID NOs: 1 to 5 comprising at least one nucleic acid according to any of the claims 19 to 23.

30. A method according to any one of claims 1 to 3, 7 and 8 comprising the following steps:

a) obtaining a biological sample containing genomic DNA,

b) extracting the genomic DNA,

c) converting cytosine bases in the genomic DNA sample which are unmethylated at the 5-position, to uracil or another base which is dissimilar to cytosine in terms of base pairing behaviour,

d) amplifying at least one fragment of the pretreated genomic DNA, wherein said fragments comprise one or more sequences selected from the group consisting of SEQ ID NOs: 6 to 25 and sequences complementary thereto, and

e) determining the methylation status of one or more genomic CpG dinucleotides by analysis of the amplificate nucleic acids.

31. A method according to any one of claims 3 to 6 comprising the following steps:

a) obtaining a biological sample containing genomic DNA,

b) extracting the genomic DNA,

c) converting cytosine bases in the genomic DNA sample which are unmethylated at the 5-position, to uracil or another base which is dissimilar to cytosine in terms of base pairing behaviour,

d) amplifying at least one fragment of the pretreated genomic DNA, wherein said fragments comprise one or more sequences selected from the group consisting of SEQ ID NOs: 14, 15, 24 and 25 and sequences complementary thereto, and

e) determining the methylation status of one or more genomic CpG dinucleotides by analysis of the amplificate nucleic acids.

32. The method as recited in claims 30 or 31, characterised in that step e) is carried out by means of hybridisation of at least one oligonucleotide according to claims 19 or 20.

33. The method as recited in claims 30 or 31, characterised in that step e) is carried out by means of hybridisation of at least one oligonucleotide according to claims 19 or 20 and extension of said hybridised oligonucleotide(s) by at least one nucleotide base.

34. The method as recited in claims 30 or 31, characterised in that step e) is carried out by means of sequencing.

35. The method as recited in claims 30 or 31, characterised in that step d) is carried out using methylation specific primers.

36. The method as recited in claim 30, further comprising in step d) the use of at least one nucleic acid molecule or peptide nucleic acid molecule comprising in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridises under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOs: 6 to 25, and complements thereof, wherein said nucleic acid molecule or peptide nucleic acid molecule suppresses amplification of the nucleic acid to which it is hybridised.

37. The method as recited in claim 31, further comprising in step d) the use of at least one nucleic acid molecule or peptide nucleic acid molecule comprising in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridises under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOs: 14, 15, 24 and 25, and complements thereof, wherein said nucleic acid molecule or peptide nucleic acid molecule suppresses amplification of the nucleic acid to which it is hybridised.

38. The method as recited in claims 30 or 31, characterised in that step e) is carried out by means of a combination of at least two of the methods described in claims 32 to 37.

39. The method as recited in claims 30 or 31, characterised in that the treatment is carried out by means of a solution of a bisulfite, hydrogen sulfite or disulfite.

40. A method according to any one of claims 1 to 16 comprising the following steps:
    a) obtaining a biological sample containing genomic DNA,
    b) extracting the genomic DNA,
    c) digesting the genomic DNA comprising one or more of the sequences from the group consisting of SEQ ID NOs: 1 to 5 and sequences complementary thereto with one or more methylation sensitive restriction enzymes, and
    d) determining of the DNA fragments generated in the digest of step c).

41. A method according to claim 40, wherein the DNA digest is amplified prior to step d).

42. The method as recited in any one of claims 30 to 39 and 41, characterised in that more than six different fragments having a length of 100 - 200 base pairs are amplified.

43. The method as recited in any one of claims 30 to 39, 41 and 42, characterised in that the amplification of several DNA segments is carried out in one reaction vessel.

44. The method as recited in any one of claims 30 to 39, 41 to 43, characterised in that the polymerase is a heat-resistant DNA polymerase.

45. The method as recited in any one of claims 30 to 39, 41 to 44, characterised in that the amplification is carried out by means of the polymerase chain reaction (PCR).

46. The method as recited in any one of claims 30 to 39 and 41 to 45, characterised in that the amplificates carry detectable labels.

47. The method according to claim 46, wherein said labels are fluorescence labels, radionuclides and/or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer.

48. The method as recited in any one of claims 30 to 39 and 41 to 45, characterised in that the amplificates or fragments of the amplificates are detected in the mass spectrometer.

49. The method as recited in any one of the claims 47 and 48, characterised in that the produced fragments have a single positive or negative net charge for better detectability in the mass spectrometer.

50. The method as recited in any one of claims 47 and 48, characterised in that detection is carried out and visualised by means of matrix assisted laser desorption/ionisation mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

51. The method as recited in any one of the claims 1 to 16 or any one of the claims 30 to 50, characterised in that the genomic DNA is obtained from cells or cellular components which contain DNA or sources of DNA comprising, for example, cell lines, histological slides, biopsies, tissue embedded in paraffin, breast tissues, blood, plasma, lymphatic fluid, lymphatic tissue, duct cells, ductal lavage fluid, nipple aspiration fluid and combinations thereof.

52. The method as recited in any one of the claims 1 to 16 or any one of the claims 30 to 50, characterised in that said biological sample is or is derived from cell lines, histological slides, biopsies, tissue embedded in paraffin, breast tissues, blood, plasma, lymphatic fluid, lymphatic tissue, duct cells, ductal lavage fluid, nipple aspiration fluid and combinations thereof.

53. A kit comprising a bisulfite (= disulfite, hydrogen sulfite) reagent as well as oligonucleotides, PNA-oligomers and/or sets of oligomers or oligonucleotides according to any one of the claims 19 to 23.

54. A kit according to claim 53, further comprising standard reagents for performing a methylation assay from the group consisting of MS-SNuPE, MSP, Methyl light, Heavy Methyl, nucleic acid sequencing and combinations thereof.

55. The use of a method according to any one of claims 1 to 17 and 30 to 51, a nucleic acid according to claim 18, of an oligonucleotide or PNA-oligomer or a set thereof according to any one of claims 19 to 23, of a kit according to claim 53 or 54, of an arrangement or an array according to any one of claims 26 to 29 or of a method of manufacturing an array according to claim 25 in the prognosis, diagnosis, treatment, characterisation, classification and/or differentiation of breast cell proliferative disorders.

56. The use of claim 55, wherein said treatment is a hormonal/antihormonal treatment.

57. The use of claim 56, wherein said hormonal/antihormonal treatment is a tamoxifen treatment.